Effects of Dietary Myostatin Inhibitory Peptide Supplementation on Growth Performance, Body Composition, Serum Biochemical Indices, and Hepatic and Serum Immune Indices in Sea Bass: Postprint

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Abstract

This experiment aimed to investigate the effects of dietary supplementation of myostatin inhibitory peptide on growth performance, body composition, serum biochemical indices, and hepatic and serum immune indices of sea bass. Juvenile sea bass with an initial average weight of (9.05$±$0.05) g were used as experimental animals. After a 1-week acclimation, 480 healthy fish of uniform size were selected and randomly divided into 4 groups with 4 replicates (aquaria) per group, with 30 fish stocked per aquarium. The four groups of experimental fish were fed four experimental diets containing myostatin inhibitory peptide at supplementation levels of 0 (control), 0.25%, 0.50%, and 0.75% for 135 days. The results showed: 1) The weight gain rate (WGR), specific growth rate (SGR), and survival rate (SR) of the 0.50% group were significantly higher than those of the control group (P<0.05). The feed conversion ratio (FCR) of all supplementation groups was significantly lower than that of the control group (P<0.05), with the 0.50% group having the lowest FCR; 2) The crude lipid content in dorsal muscle of all supplementation groups was significantly lower than that of the control group (P<0.05). The crude protein content in dorsal muscle of the 0.50% group was significantly higher than that of the control group (P<0.05). There were no significant differences among groups in moisture, crude protein, crude lipid, and crude ash contents of whole fish, nor in moisture and crude ash contents of dorsal muscle (P>0.05); 3) The contents of serum total protein (TP), albumin (ALB), and total cholesterol (TC) in the 0.25% group were significantly lower than those in other groups (P<0.05). There was no significant difference in serum triglyceride (TG) content among groups (P>0.05); 4) The serum alkaline phosphatase (ALP) activity of the 0.50% and 0.75% groups was significantly

higher than that of the control group (P<0.05). The hepatic lysozyme (LZM) activity of the 0.25% and 0.50% groups was significantly higher than that of the control group (P<0.05). There were no significant differences among groups in total antioxidant capacity (T-AOC) of liver and serum, or in hepatic superoxide dismutase (SOD) activity (P>0.05). In conclusion, dietary supplementation of myostatin inhibitory peptide can promote growth and enhance immune capacity of sea bass, with the optimal supplementation level being 0.50% under the conditions of this experiment.

Full Text

Effects of Dietary Myostatin Inhibitory Peptides on Growth Performance, Body Composition, Serum Biochemical Indices, and Liver and Serum Immune Indices of Sea Bass (Lateolabrax japonicus)

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Abstract

This study investigated the effects of dietary myostatin (MSTN) inhibitory peptides on growth performance, body composition, serum biochemical indices, and liver and serum immune indices of sea bass (Lateolabrax japonicus). Juvenile sea bass with an average initial body weight of (9.05$±$0.05) g were acclimated for one week, after which 480 healthy fish of uniform size were randomly allocated to 4 groups with 4 replicates per group (30 fish per aquarium). The four groups were fed experimental diets supplemented with 0% (control), 0.25%, 0.50%, and 0.75% MSTN inhibitory peptides for 135 days. The results showed: (1) The weight gain rate (WGR), specific growth rate (SGR), and survival rate (SR) of the 0.50% group were significantly higher than those of the control group (P<0.05), while the feed conversion ratio (FCR) of all supplementation groups was significantly lower than that of the control group (P<0.05), with the 0.50% group showing the lowest FCR. (2) No significant differences were observed among groups in whole-body moisture, crude protein, crude lipid, or ash content (P>0.05). However, dorsal muscle crude lipid content in all supplementation groups was significantly lower than in the control group (P<0.05), and dorsal muscle crude protein content in the 0.50% group was significantly higher than in the control group (P<0.05). (3) Serum total protein (TP), albumin (ALB), and total cholesterol (TC) contents in the 0.25% group were significantly lower than those in other groups (P<0.05), while no significant differences were found in serum triglyceride (TG) content among groups (P>0.05). (4) Serum alkaline phosphatase (ALP) activity in the 0.50% and 0.75% groups was significantly higher than in the control group (P<0.05), and liver lysozyme

(LZM) activity in the 0.25% and 0.50% groups was significantly higher than in the control group ($P<0.05$). No significant differences were detected in liver or serum total antioxidant capacity (T-AOC) or liver superoxide dismutase (SOD) activity among groups ($P>0.05$). In conclusion, dietary MSTN inhibitory peptides can promote growth and enhance immune capacity in sea bass, with an optimal supplementation level of 0.50% under the conditions of this experiment.

Keywords: MSTN inhibitory peptides; sea bass (Lateolabrax japonicus); growth performance; serum biochemical indices; immune indices

Introduction

Myostatin (MSTN), also known as growth differentiation factor 8 (GDF-8), is a negative regulator of muscle cell growth in animals and belongs to the transforming growth factor β (TGF-β) superfamily. In fish, MSTN is widely distributed and expressed in various tissues including muscle, brain, gills, intestine, kidney, and gonads. Studies have reported that MSTN gene expression in white bass (Morone chrysops) occurs primarily in muscle tissue. MSTN consistently functions as a negative regulator of muscle growth, with its primary role in fish being similar to that in mammals. The regulatory mechanism involves MSTN acting as an extracellular signaling molecule that binds to receptors on myoblast membranes, triggering receptor autophosphorylation and initiating intracellular signal transduction pathways that target the regulatory regions of myogenic determination factor (MyoD) genes, thereby modulating muscle protein gene expression. Research has demonstrated that MSTN gene knockout in mice results in myocyte hyperplasia and hypertrophy, with significantly increased muscle mass compared to normal mice. Moreover, suppressing MSTN expression in adult mice can increase muscle mass by approximately 25%.

For decades, it was believed that proteins must be hydrolyzed into free amino acids before utilization, until Newey and colleagues in the 1960s provided evidence that peptides can be absorbed intact. Small peptides are compounds consisting of two or more amino acids connected by peptide bonds and possess various biological functions. Compared to amino acid absorption, small peptide absorption is characterized by faster transport, lower energy consumption, lack of saturation, and absence of competition or inhibition among different peptides. Numerous studies have shown that dietary small peptides can promote animal growth, improve feed efficiency, enhance immunity, and regulate meat flavor. For instance, Kotzamanis et al. found that adding 10% small peptides to feed produced optimal growth promotion in European sea bass (Dicentrarchus labrax), while Yu et al. demonstrated that casein-derived small peptides could promote growth and feed utilization in grass carp (Ctenopharyngodon idella).

As the most potent known inhibitor of muscle growth, MSTN has been the target of extensive research seeking inhibitors or related technologies to block its function. Reports indicate that MSTN inhibitors can reduce its negative

regulation of muscle growth and development, leading to increased muscle mass. Whittemore et al. injected MSTN antibodies into mice and observed increased muscle mass and body weight. Zhang et al. prepared MSTN antibodies and microinjected them into the yolk region of fertilized eggs of largemouth bass (Micropterus salmoides), resulting in promoted larval growth. MSTN inhibitory peptides represent a novel class of small peptides that function similarly to MSTN antibodies by inhibiting MSTN activity. This study used sea bass as the experimental animal to investigate the effects of different dietary levels of MSTN inhibitory peptides on growth performance, body composition, serum biochemical indices, and liver and serum immune indices, aiming to provide a theoretical basis for basic research and practical application in sea bass formulated feed development.

1.1 Experimental Diets

Four isonitrogenous and isolipidic experimental diets were formulated (approximately 40.0% crude protein and 6.5% crude lipid), with composition and nutrient levels shown in . The diets were supplemented with MSTN inhibitory peptides (provided by Gonglin Industrial Co., Ltd., Shenzhen) at levels of 0% (control), 0.25%, 0.50%, and 0.75%. All feed ingredients were ground and passed through a 40-mesh sieve before being weighed according to the proportions in and initially mixed. The mixture was blended in a commercial feed mixer for 15 minutes, after which pre-mixed fish oil, soybean oil, and lecithin were added and blended for an additional 15 minutes. Approximately 40% distilled water (v/w) was then added, and the mixture was blended for another 15 minutes before being extruded into 2.0 mm and 3.5 mm pellets using a twin-screw extruder (developed by the Institute of Mechanical Engineering, South China University of Technology). The pellets were polished and air-dried in an air-conditioned room to approximately 10% moisture content, then packaged in sealed plastic bags, labeled, and stored at -20°C until use.

1.2 Experimental Animals and Husbandry

The feeding trial was conducted at the Zhuhai Fish and Shrimp Nutrition Research Base. Juvenile sea bass with an average initial body weight of (9.05$±$0.05) g were acclimated for one week in 1000 L concrete tanks and fed commercial feed (Guangdong Junyou Feed Co., Ltd. No. 1 juvenile shrimp feed, containing $ $41.0% crude protein and $ $4.0% crude lipid) during this period. After acclimation, 480 healthy fish of uniform size were selected and stocked into 16 aquaria (250 L capacity, 200 L water volume) at 30 fish per aquarium. Each experimental diet was randomly assigned to 4 aquaria. Fish were fed twice daily (08:30 and 18:00) with 2.0 mm pellets during weeks 1-4 and 3.5 mm pellets thereafter. The system operated with micro-flow water exchange. Initial feeding rate was set at 6% of body weight and adjusted based on feeding response, with daily records maintained. Uneaten feed and feces were removed

daily, and uneaten feed was collected, dried, and weighed. The trial lasted 135 days. Water quality parameters were monitored regularly: temperature 27-31°C, salinity 6-7‰, dissolved oxygen 6-7 mg/L, pH 7.0-7.4, sulfate 0-0.05 mg/L, nitrite nitrogen 0.05-0.10 mg/L, and ammonia nitrogen 0.20-0.40 mg/L.

1.3 Sample Collection

During the trial, feeding behavior, mortality, and response to external stimuli were recorded. After 135 days, fish were fasted for 24 hours before final weighing and counting in each aquarium to calculate weight gain rate (WGR), feed conversion ratio (FCR), survival rate (SR), and specific growth rate (SGR). Two fish were randomly selected from each aquarium as whole-body samples. An additional five fish per aquarium were measured for body length and weight to calculate condition factor (CF). Blood was collected from the caudal vein using a 2 mL syringe rinsed with anticoagulant, allowed to clot for 2 hours, then centrifuged at 4,000 rpm for 10 minutes at 4°C. Serum was aliquoted into 1.5 mL tubes for biochemical and immune index analysis. After blood collection, fish were dissected to separate viscera, liver, and intraperitoneal fat for calculating viscerosomatic index (VSI), hepatosomatic index (HSI), and intraperitoneal fat ratio (IPF). Liver samples were stored in 1.5 mL tubes, snap-frozen in liquid nitrogen, and stored for immune index analysis. Dorsal muscle was collected for composition analysis.

Liver tissue homogenate preparation: Frozen liver samples (0.2-0.4 g) stored at -80°C were thawed on ice, rinsed in cold physiological saline to remove blood, blotted dry, weighed, and placed in 10 mL tubes. Cold 0.86% physiological saline was added at a 1:9 ratio (w/v), and samples were homogenized using an automatic sample grinder (JXFSTPRP-24, Shanghai Jingxin Industrial Development Co., Ltd.). The homogenate was centrifuged at 2,500 rpm for 10 minutes at 4°C, and the supernatant was aliquoted into 1.5 mL tubes and stored at -80°C for liver immune index analysis.

1.4.1 Conventional Nutrient Analysis

Feed, whole-body, and dorsal muscle samples were dried at 105°C to constant weight for moisture determination. Dried samples were ground and analyzed for crude protein (Kjeldahl method using 1030-Auto-analyzer, Tecator AB, Höganäs, Sweden), crude lipid (Soxhlet extraction using HT6, Tecator AB, Höganäs, Sweden), and ash content (muffle furnace at 550°C).

1.4.2 Serum Biochemical and Liver/Serum Immune Indices

Serum samples were sent to the First Affiliated Hospital of Sun Yat-sen University for determination of total protein (TP), albumin (ALB), total cholesterol (TC), triglyceride (TG) contents, and alkaline phosphatase (ALP) activity using a Hitachi 7107A automatic biochemical analyzer.

Serum total antioxidant capacity (T-AOC), liver T-AOC, superoxide dismutase (SOD) activity, lysozyme (LZM) activity, and liver protein content were determined using assay kits provided by Nanjing Jiancheng Bioengineering Institute.

1.5 Calculation Formulas

Weight gain rate (%) = 100 × (final body weight - initial body weight) / initial body weight
Specific growth rate (%/d) = 100 × (ln final body weight - ln initial body weight) / experimental days
Survival rate (%) = 100 × final fish number / initial fish number
Feed conversion ratio = (feed fed dry weight - uneaten feed dry weight) / (final body weight - initial body weight)
Condition factor (g/cm^3) = 100 × body weight (g) / body length (cm)^3
Hepatosomatic index (%) = 100 × liver weight / body weight
Intraperitoneal fat ratio (%) = 100 × intraperitoneal fat weight / body weight
Viscerosomatic index (%) = 100 × viscera weight / body weight

1.6 Statistical Analysis

Results are expressed as mean ± standard deviation. Data were analyzed using one-way ANOVA with SPSS 21.0 software. When significant differences were detected, Duncan' s multiple range test was used for inter-group comparisons. Statistical significance was set at $P<0.05$.

2.1 Effects of Dietary MSTN Inhibitory Peptides on Growth Performance of Sea Bass

As shown in , the final body weight, weight gain rate, and specific growth rate of the 0.50% group were significantly higher than those of the control and 0.25% groups ($P<0.05$), but did not differ significantly from the 0.75% group ($P>0.05$). The weight gain rate of the 0.50% group was 24.9% higher than that of the control group. Survival rate in the 0.50% group was significantly higher than in the control and 0.75% groups ($P<0.05$), but not significantly different from the 0.25% group ($P>0.05$). The feed conversion ratio of the 0.50% group was significantly lower than all other groups ($P<0.05$). No significant differences were observed among groups in morphological indices including condition factor, viscerosomatic index, hepatosomatic index, and intraperitoneal fat ratio ($P>0.05$).

2.2 Effects of Dietary MSTN Inhibitory Peptides on Body Composition of Sea Bass

As shown in , no significant differences were found among groups in whole-body moisture, crude protein, crude lipid, or ash content ($P>0.05$). Similarly,

dorsal muscle moisture and ash content did not differ significantly among groups (P>0.05). However, dorsal muscle crude protein content in the 0.50% group was significantly higher than in all other groups (P<0.05), while dorsal muscle crude lipid content in all three supplementation groups was significantly lower than in the control group (P<0.05).

2.3 Effects of Dietary MSTN Inhibitory Peptides on Serum Biochemical Indices of Sea Bass

As shown in , serum total protein, albumin, and total cholesterol contents in the 0.25% group were significantly lower than those in other groups (P<0.05), with no significant differences among the other groups (P>0.05). Serum triglyceride content showed a decreasing trend with increasing MSTN inhibitory peptide supplementation, but differences among groups were not significant (P>0.05).

2.4 Effects of Dietary MSTN Inhibitory Peptides on Liver and Serum Immune Indices of Sea Bass

As shown in , no significant differences were observed among groups in liver or serum total antioxidant capacity or liver superoxide dismutase activity (P>0.05). Liver lysozyme activity in the 0.25% and 0.50% groups was significantly higher than in the control and 0.75% groups (P<0.05). Serum alkaline phosphatase activity showed an overall increasing trend with MSTN inhibitory peptide supplementation, with the 0.50% and 0.75% groups being significantly higher than the control and 0.25% groups (P<0.05).

3.1 Effects of Dietary MSTN Inhibitory Peptides on Growth Performance of Sea Bass

The results indicate that dietary supplementation with 0.50% MSTN inhibitory peptides benefits sea bass growth, improves survival rate, and reduces feed conversion ratio. Literature review reveals minimal research on MSTN inhibitors in aquafeeds, with most studies focusing on immunoneutralization approaches that utilize immunological principles to neutralize endogenous MSTN through active or passive immunization, thereby inactivating its biological function and promoting muscle growth and development. Studies have shown that MSTN antibody injection in mice, largemouth bass fertilized eggs, and broiler chicken yolk can increase skeletal muscle mass and promote growth. Tang et al. reported that mice injected with MSTN DNA vaccine showed significantly increased muscle mass and strength. Duchenne muscular dystrophy model mice exhibited increased body weight, muscle mass, and muscle volume after intraperitoneal MSTN antibody injection for three months, and adult rats showed increased muscle mass following MSTN antibody administration. Other studies have

demonstrated that silencing MSTN transcripts during early embryonic development in zebrafish promoted muscle growth and caused muscle hyperplasia or hypertrophy. Transgenic mice overexpressing a dominant-negative MSTN that specifically binds to activin receptor protein (ActR) IIB showed dramatic muscle mass increases comparable to MSTN knockout mice. These findings collectively indicate that MSTN inhibitors promote growth and increase muscle mass, consistent with our results showing that 0.5% MSTN inhibitory peptide supplementation enhanced fish growth. Furthermore, as small peptides, MSTN inhibitory peptides share characteristics with other peptide additives that have demonstrated clear growth-promoting effects in aquatic animals. For example, Jiang found that 0.75% and 1.00% small peptide supplementation significantly improved weight gain rate, feeding rate, and specific growth rate in juvenile starry flounder (Platichthys stellatus), while Liu et al. reported that 6% Leneng small peptide supplementation in Pacific white shrimp (Litopenaeus vannamei) diets enhanced growth rate, feed conversion efficiency, and survival rate. Based on available literature, the growth-promoting effects of MSTN inhibitory peptides in sea bass may be attributed to several mechanisms. First, as small peptides, they are absorbed rapidly with low energy cost and without carrier saturation, thereby enhancing amino acid absorption and improving dietary protein utilization efficiency. Second, small peptides can promote intestinal peristalsis and improve digestive function. Third, MSTN inhibitory peptides suppress MSTN activity during muscle development, reducing MSTN' s inhibitory effects on myoblast proliferation and differentiation, thereby decreasing its negative regulation of muscle growth and increasing muscle mass. In this study, 0.50% MSTN inhibitory peptide supplementation yielded the best growth performance, while higher levels (0.75%) did not show further improvement. However, with only three supplementation levels tested, these results cannot definitively indicate whether levels above 0.50% would fail to promote growth or potentially inhibit it. Future studies should examine higher supplementation levels to determine their effects on growth and feed utilization.

3.2 Effects of Dietary MSTN Inhibitory Peptides on Body Composition of Sea Bass

The results demonstrate that the 0.50% group had significantly higher dorsal muscle crude protein content, while all supplementation groups showed significantly lower dorsal muscle crude lipid content compared to the control group. These findings align with Yue et al., who reported that MSTN gene deficiency leads to increased muscle mass and decreased fat deposition. McPherron et al. confirmed that 9-10-month-old MSTN knockout mice had 20% less adipose tissue than normal mice. Similar results have been reported for small peptide supplementation in juvenile grass carp. Jiang' s study showed that 1.0% and 1.5% small peptide supplementation significantly increased dorsal muscle crude protein content in starry flounder, while the highest dorsal muscle crude lipid content occurred in the control group without peptide supplementation. The increased dorsal muscle protein content can be explained by the same mecha-

nisms promoting overall growth. The reduced dorsal muscle lipid content may be attributed to four factors: (1) small peptide additives enhance protein synthesis, reducing the diversion of amino acids to lipid synthesis and decreasing fat accumulation; (2) MSTN deficiency inhibits adipocyte development and promotes lipid metabolism; (3) increased muscle mass raises resting energy expenditure, thereby reducing fat deposition; and (4) the absence of MSTN may affect preadipocyte formation or triglyceride synthesis in adipocytes.

3.3 Effects of Dietary MSTN Inhibitory Peptides on Serum Biochemical Indices of Sea Bass

Serum total protein content reflects protein metabolism status and can be used to evaluate protein and amino acid utilization efficiency. Serum albumin serves as both a nutrient carrier and a protein source for maintaining blood osmotic pressure, providing energy, and tissue repair. In this study, the 0.25% group showed significantly lower serum total protein and albumin contents than other groups, which contradicts previous research. Studies have reported that dietary small peptides promote serum protein synthesis and increase globulin levels, thereby enhancing immunity. However, the 0.50% and 0.75% groups showed no significant differences from the control group, suggesting that the supplementation levels in this study may have been insufficient to significantly enhance serum protein synthesis in sea bass, with lower levels potentially even reducing synthesis. The specific mechanisms require further investigation.

Total cholesterol and triglyceride levels reflect fat accumulation status. The 0.25% group exhibited significantly lower serum total cholesterol content than other groups, consistent with Jiang' s report that small peptide supplementation reduced serum total cholesterol in starry flounder and McPherron' s finding that MSTN knockout mice had lower blood cholesterol than normal mice. This may be due to MSTN inhibitory peptides promoting lipid metabolism and reducing serum lipid levels. Although serum triglyceride content was lower in all supplementation groups compared to the control, differences were not significant, suggesting that the supplementation levels may have been insufficient to significantly reduce serum triglyceride content in sea bass.

3.4 Effects of Dietary MSTN Inhibitory Peptides on Liver and Serum Immune Indices of Sea Bass

Serum alkaline phosphatase activity serves as an effective indicator of phosphorus sufficiency and reflects calcium and phosphorus digestion. When fish require more calcium for growth, alkaline phosphatase can catalyze calcium release from bone tissue to meet physiological demands. Therefore, increased alkaline phosphatase activity within certain limits benefits fish growth and skeletal development. In this study, all supplementation groups showed higher serum alkaline phosphatase activity than the control group, with the 0.50% group showing the highest activity, corresponding to the improved growth performance.

Total antioxidant capacity of the defense system is closely related to health status and includes enzymatic and non-enzymatic antioxidant systems. The former relies on antioxidant enzymes such as catalase and glutathione peroxidase, while the latter depends on metalloproteins, amino acids, and vitamins. Superoxide dismutase specifically catalyzes toxic superoxides produced by metabolism or environmental stress, converting them to hydrogen peroxide and oxygen, which are then further metabolized by other peroxidase system components to water and oxygen, ensuring normal physiological function. The antioxidant defense system operates through three pathways: (1) eliminating reactive oxygen species and free radicals to inhibit excessive lipid oxidation; (2) decomposing peroxides to break peroxidation chains; and (3) removing catalytic metal ions. In this study, MSTN inhibitory peptide supplementation did not significantly affect liver total antioxidant capacity, superoxide dismutase activity, or serum total antioxidant capacity, indicating limited effects on antioxidant capacity.

Lysozyme is an important non-specific immune factor produced by macrophages and neutrophils in fish, playing a crucial defensive role by hydrolyzing bacterial cell walls and inducing the synthesis and secretion of other immune factors. This study found that 0.25% and 0.50% MSTN inhibitory peptide supplementation significantly increased liver lysozyme activity compared to the control group. Elevated lysozyme activity can induce other immune factors to participate in immune defense, thereby enhancing immunity. Additionally, MSTN inhibitory peptides may improve immune activity by generating immune-active peptides (such as immunostimulatory and antimicrobial peptides) through hydrolysis, which ultimately participate in immune system regulation.

In conclusion, under the conditions of this experiment, the optimal dietary supplementation level of MSTN inhibitory peptides for sea bass is 0.50%.

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Note: Figure translations are in progress. See original paper for figures.